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# A morphometric study of the rectocoprodeal sphincter in the domestic duck

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## INTRODUCTION

The caudal part of the rectum in birds opens into the cranial compartment of the cloaca, the coprodeum. There is considerable debate as to whether or not an anatomical boundary exists at the junction of the rectum and coprodeum (for a review of the literature see King, 1981). Most observations have been concerned with the presence of a rectocoprodeal fold or changes in the appearance of the mucosa, and little attention has been given to the existence of a sphincter which might act to close off the rectum from the coprodeum. Such a sphincter could play a very important part in regulating the movement of ingesta and urine between the various parts of the hindgut (Skadhauge, 1973; Duke, Evanson & Huberty, 1979). Using morphometric techniques it is now possible to obtain precise evidence for the existence of sphincters, even when the thickening of the circular muscle is very slight or non-existent (Cai & Gabella, 1984; Vaithilingham, Wong & Ling, 1984; Mahdi & McLelland, 1988). The purpose of the present study, therefore, is to investigate the presence of a rectocoprodeal sphincter in the domestic duck (*Anas platyrhynchos*) by means of quantitative methods which establish the relative density of the innervation and the predominant type of axon profile.

## MATERIALS AND METHODS

### *Light microscopy*

Fourteen adult domestic ducks were killed by an overdose of sodium pentobarbitone. After death the region of the rectocoprodeal junction was removed, ligated 2·5 cm on either side of the junction, and injected with fixative at a pressure of 10 mmHg. Tissue was fixed with either 10% formal saline for seven days (seven birds) or Bouin's fluid for 24 hours (seven birds). 7 $\mu$m thick, serial transverse and longitudinal paraffin sections were stained with either haematoxylin and eosin or Masson's trichrome.

### *Transmission electron microscopy*

Small blocks of tissue from ten birds were fixed with 6% glutaraldehyde in 0·1 M sodium cacodylate at pH 7·3 for $2\frac{1}{2}$ hours, postfixed in osmium tetroxide, and embedded in Araldite. The regions to be studied with the electron microscope were first identified by the light microscope in semithin sections stained with toluidine blue. Ultrathin sections were stained with lead citrate and uranyl acetate and examined in a Philips EM400 electron microscope.

A quantitative study of the nerve bundles in the circular muscle layer was carried

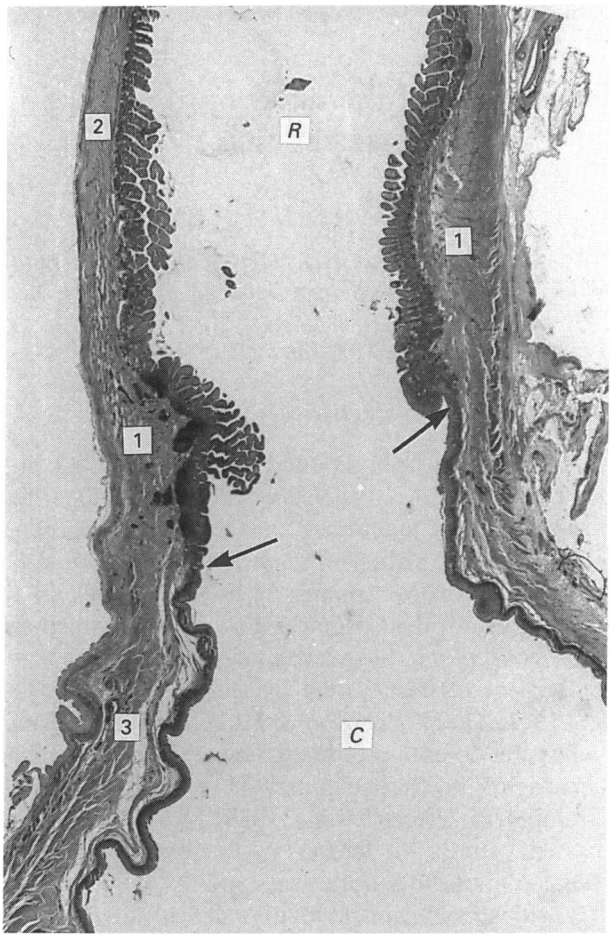


Fig. 1. Light micrograph of a longitudinal section of the rectocoprodeal junction of the domestic duck, *C*, coprodeum; *R*, rectum. The dorsal wall of the rectum and coprodeum is on the right hand side of the photograph. The oblique junction between the rectum and coprodeum where there is a change in the mucosa is indicated by arrows. The quantitative study of the nerve bundles in the circular muscle was carried out at the thickening of the muscle in the rectum (1) 1–2 mm from the rectocoprodeal junction, and at the rectum (2) and coprodeum (3) 5 mm from the junction. Masson's trichrome. ×8.

out on the areas shown in Figure 1, namely the rectum 1–2 mm from the rectocoprodeal junction and the rectum and coprodeum 5 mm from the junction. Ultrathin sections were photographed in the electron microscope at ×1750, and a photographic montage prepared from prints at a magnification of ×5500. Each intramuscular nerve bundle in the montage was rephotographed at a magnification of ×10000 and final prints were prepared at a magnification of ×20000. The number of nerve bundles and axon profiles, and the percentages of non-vesiculated and vesiculated axon profiles per number of circular muscle cell profiles were counted and subjected to statistical analysis.

## RESULTS

The point of transition from the rectum to the coprodeum was marked grossly by an obliquely orientated irregular line, the line on the dorsal wall of the gut lying cranial

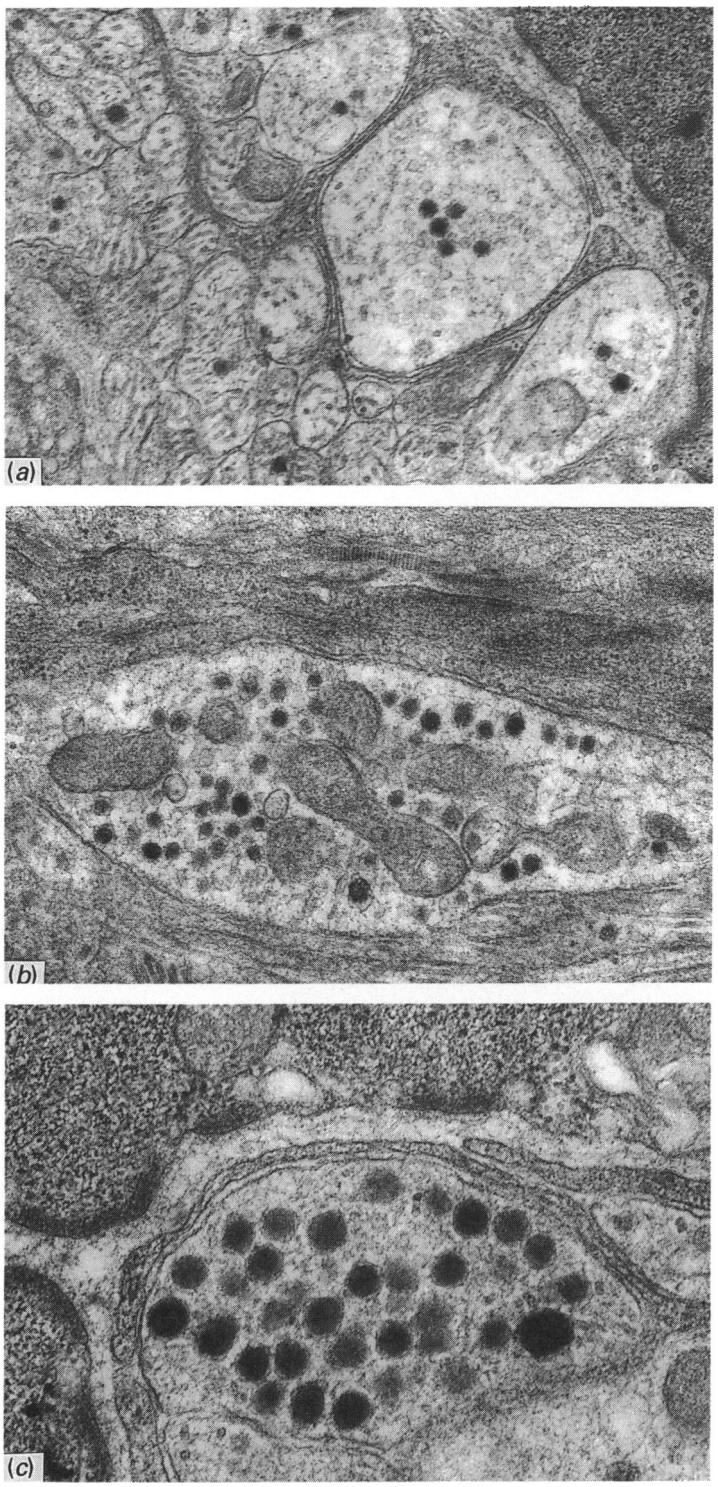


Fig. 2(*a–c*). Transmission electron micrographs of axon profiles in the circular muscle layer at the rectocoprodeal junction of the domestic duck. In (*a*) the axon profiles contain many agranular vesicles and a few large granular vesicles. In (*b*) the axon profile contains many agranular and small granular vesicles and a few large granular vesicles. In (*c*) the axon profile contains many agranular and large granular vesicles. (*a*) ×37500; (*b*) ×35000; (*c*) ×60000.

Table 1. *Distribution of nerves and axon profiles in the circular muscle in the region of the rectocoprodeal junction of the domestic duck*

| Region | No. of circular muscle fibres | No. of nerves | Total no. of axon profiles | Statistical significance (*t* test) | No. of non-vesiculated axon profiles | No. of vesiculated axon profiles | Statistical significance (*t* test) |
|---|---|---|---|---|---|---|---|
| Rectum 5 mm from junction | 1080 | 23 | 339 (100%) | $t = 5{\cdot}79$ ($P < 0{\cdot}01$) | 284 (83·8%) | 55 (16·2%) | $t = 11{\cdot}6$ ($P < 0{\cdot}01$) |
| Rectum 1–2 mm from junction | 1112 | 36 | 635 (100%) | $t = 5{\cdot}47$ $P < 0{\cdot}01$) | 472 (74·3%) | 163 (25·7%) | $t = 10{\cdot}6$ ($P < 0{\cdot}01$) |
| Coprodeum 5 mm from junction | 990 | 20 | 267 (100%) | | 220 (82·4%) | 47 (17·6%) | |

to that on the ventral wall. At this point there was a slight constriction in the diameter of the lumen and the light brown colour of the mucosa of the rectum changed abruptly to the white appearance of the coprodeal mucosa. A rectocoprodeal fold was not observed.

The muscle tunic of the rectum and coprodeum consisted of two closely apposed layers: an outer longitudinal layer and an inner circular layer (Fig. 1). In the distended gut the circular layer 5 mm from the rectocoprodeal junction measured 375–450 $\mu$m in the rectum and 310–395 $\mu$m in the coprodeum. One to two millimetres cranial to the rectocoprodeal junction the circular muscle of the rectum was thickened forming an obliquely orientated ring 550–625 $\mu$m thick, the dorsal part of the thickening of the muscle lying cranial to the ventral part (Fig. 1).

The nerve bundles in the circular muscle were distributed throughout the layer and usually contained between 2 and 60 axons. Larger-sized nerve bundles with 90–150 axons were only observed in the thickened muscle of the rectum 1–2 mm cranial to the rectocoprodeal junction. Three types of vesicles were identified: agranular vesicles which measured 40–70 nm in diameter; small granular vesicles measuring 45–75 nm in diameter; and large granular vesicles measuring 80–150 nm in diameter. Depending on the type of vesicle they contained, three varieties of vesiculated axon profile were observed. The first variety had numerous agranular vesicles and a few large granular vesicles (Fig. 2*a*); the second contained numerous agranular and small granular vesicles and a few larger granular vesicles (Fig. 2*b*); and the third contained numerous agranular and large granular vesicles (Fig. 2*c*). The total number of nerves and axon profiles per thousand muscle cell profiles and the number of vesiculated and non-vesiculated axon profiles in the different regions is shown in Table 1. The data indicate that the innervation of the thickened circular muscle of the rectum 1–2 mm from the rectocoprodeal junction (635 axons; 1112 muscle cells) was significantly denser ($P <$ 0·01) than that of the circular muscle 5 mm from the junction in the rectum (339 axons; 1080 muscle cells) and coprodeum (167 axons; 990 muscle cells). The number of vesiculated axon profiles and their percentage of the total in the thickened rectal muscle 1–2 mm from the rectocoprodeal junction (163; 25·7%) was significantly greater ($P < 0{\cdot}01$) than that in the circular muscle 5 mm from the junction in the rectum (55; 16·2%) and coprodeum (47; 17·6%).

## DISCUSSION

The boundary between the rectum and coprodeum in the domestic duck was identified grossly by the presence of an obliquely orientated irregular line and a change in the colour of the mucosa. However, contrary to the observations of Liebe (1914) and Komarek (1969), a true rectocoprodeal fold was not found. The present investigation has established for the first time the existence of an obliquely orientated ring-like thickening of the circular muscle of the rectum 1–2 mm cranial to the rectocoprodeal junction, although the thickening was not at all as well developed as the ileal and caecal sphincters described in the domestic duck by Mahdi & McLelland (1988). Despite its limited thickening, the muscular ring had a density of innervation and a frequency of axon profiles which were significantly higher than those in the circular muscle elsewhere. Since similar morphometric observations on the innervation of sphincters have previously been made in birds (Mahdi & McLelland, 1988) and mammals (Cai & Gabella, 1984; Vaithilingam *et al.* 1984) these quantitative data are further support for the existence in this part of the gut of a powerful sphincter. Although the sphincter anatomically belongs entirely to the rectum, its close proximity to the coprodeum suggests that functionally it would be more appropriate to refer to it as a 'rectocoprodeal' sphincter.

The present ultrastructural study has confirmed the existence of the three types of axon profile described by Mahdi & McLelland (1988), including possible adrenergic-type axons with small granular vesicles, possible cholinergic-type axons with small agranular vesicles, and possible peptidergic axons containing many large granular vesicles.

The precise role of the sphincter in avian hindgut function is not known and must await physiological investigations. It is well-established in the domestic fowl and turkey that hindgut ingesta move both aborally towards the cloaca and in an oral direction into the caeca, whilst at the same time urine is retrogradely moved from the cloaca to the caeca for reabsorption (Koike & McFarland, 1966; Akester, Anderson, Hill & Osbaldiston, 1967; Nechay, Boyarsky & Catacunan-Labay, 1968; Skadhauge, 1973; Duke *et al.* 1979). The anatomy of the large intestine of the domestic duck, in which the caeca are well-developed (McLelland, 1989) and there are thick ileal and caecal sphincters (Mahdi & McLelland, 1988) as well as a rectocoprodeal sphincter, would suggest a similar role in digestion and osmoregulation, although functional studies are still required. It is hoped that the present evidence for a sphincter will contribute to the background of anatomical information which is needed to plan and interpret such studies.

## SUMMARY

The presence of a sphincter at the junction of the rectum and coprodeum of the domestic duck (*Anas platyrhynchos*) was investigated using light microscopy and by an ultrastructural morphometric study of the axon profiles in the circular muscle. One to two millimetres cranial to the rectocoprodeal junction the circular muscle forms a slightly thickened obliquely orientated ring. The density of innervation, including the total number of axon profiles and the number of vesiculated axon profiles, is significantly higher in the thickened ring than in the circular muscle of the rectum and coprodeum 5 mm from the rectocoprodeal junction. It is concluded, therefore, that in the domestic duck a sphincter muscle, the rectocoprodeal sphincter, exists close to the junction of the rectum and coprodeum.

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